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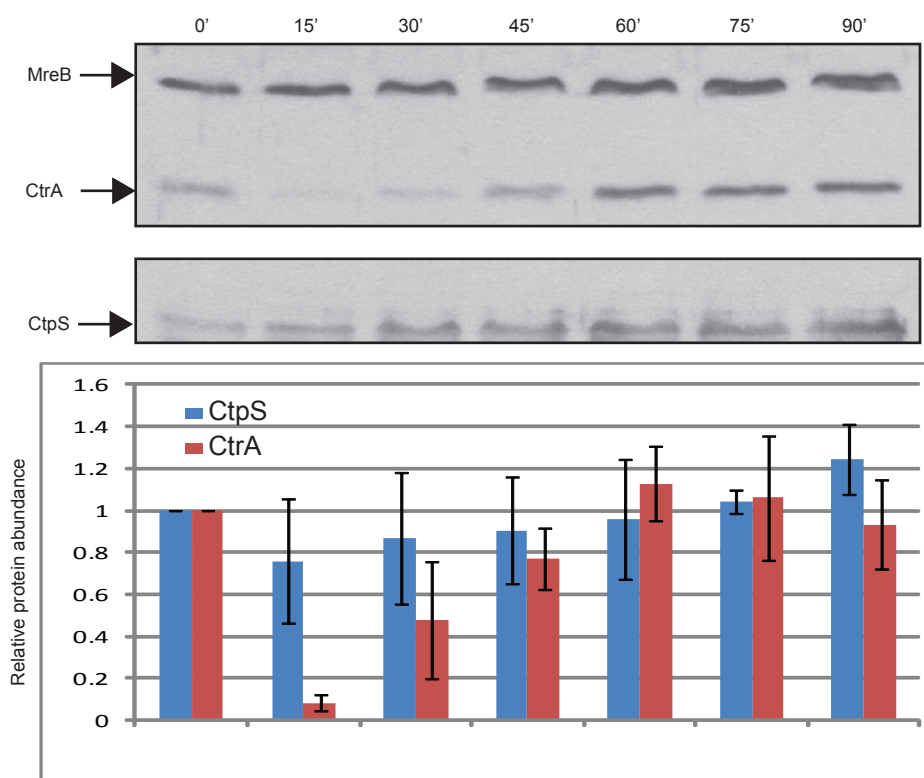


Figure S1 Abundance of *Caulobacter* CtpS throughout the cell cycle. *Caulobacter* CtpS levels throughout the cell cycle. Representative blot images shown of CtpS levels from CB15N cells collected at 15-minute intervals after synchrony. CtrA levels were monitored as an example of a cell cycle regulated protein. MreB, CtrA, and CtpS antibodies were used at 1:15,000, 1:15,000, and 1:2,500 dilutions respectively. Exposure times

for MreB and CtrA differ from CtpS. ImageJ was used to quantify CtpS, CtrA and MreB abundance from multiple experiments. Displayed below a blot images is the average of the normalized intensity of CtrA and CtpS to MreB from multiple experiments. The graph displays relative protein abundance and cannot be used to compare absolute CtrA and CtpS levels during the cell cycle. Error bars represent the standard deviation from the mean.

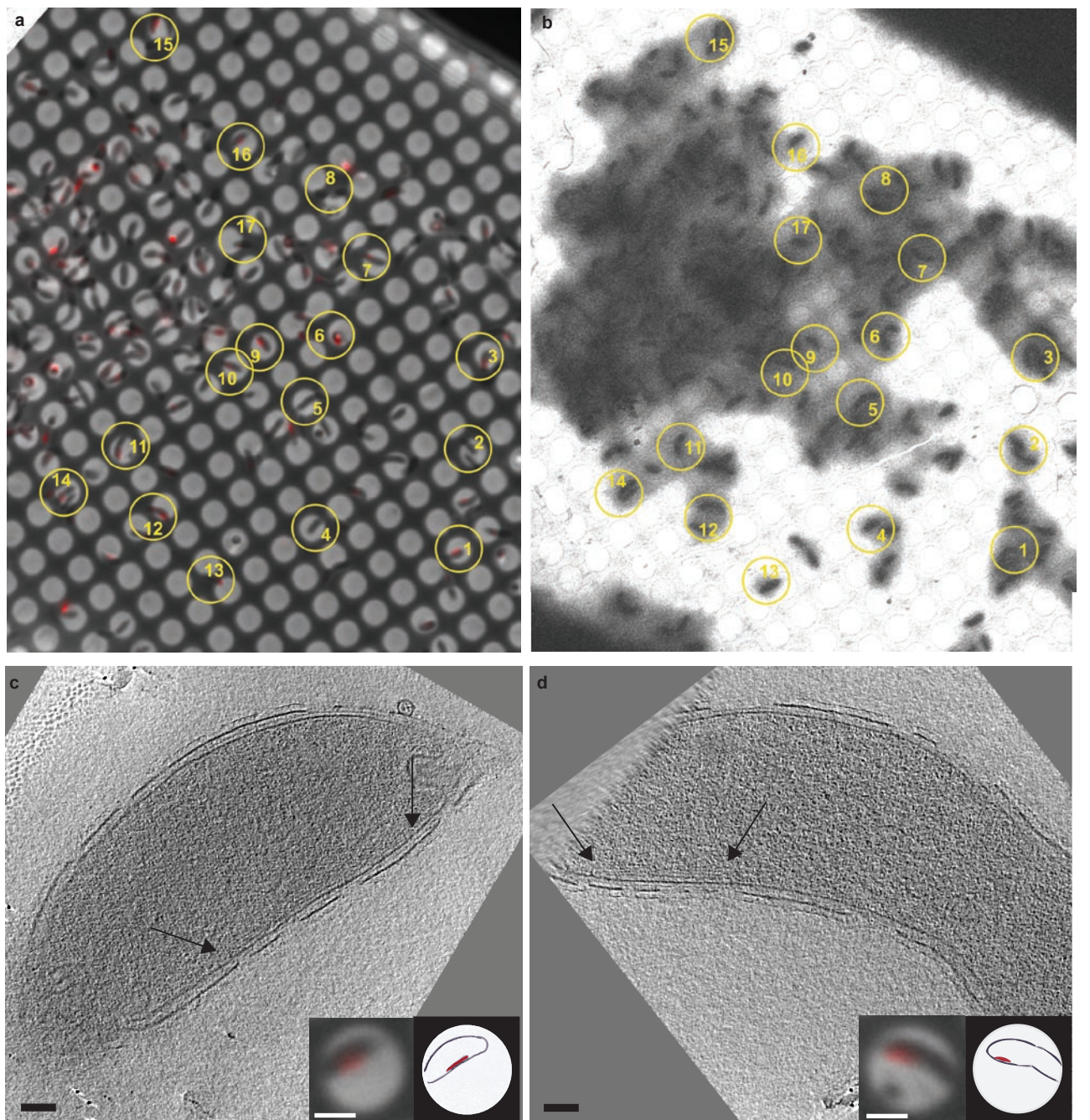


Figure S2 Light and electron microscopy images of EM grids for correlated LM-ECT. (a) Low-magnification phase/fluorescent image of an EM grid with *Caulobacter* expressing mCherry-CtpS (ZG153). (b) Electron micrograph of the same EM grid captured after light microscopy. The holes in the carbon foil are 2 μ m in diameter. The numbers reflect the numbers of the individual cells imaged. (c-d) Co-localization of mCherry-CtpS and filamentous structures in *Caulobacter*. Cells expressing mCherry-ctpS were fixed on EM

grids and imaged first by fluorescence light microscopy and then by ECT. Representative tomographic slices, phase/fluorescence overlay (left inset), and cartoon depiction (right inset) of the same cells are shown. These cells correspond to cells #16 (c) and #11 (d) from the field shown in (a). Arrows point to the ends of the filaments in the ECT images, which correspond to the positions of the mCherry-CtpS structures shown in the insets. Scale bars represent 100 nm for EM and 1 μ m for the inset LM images.

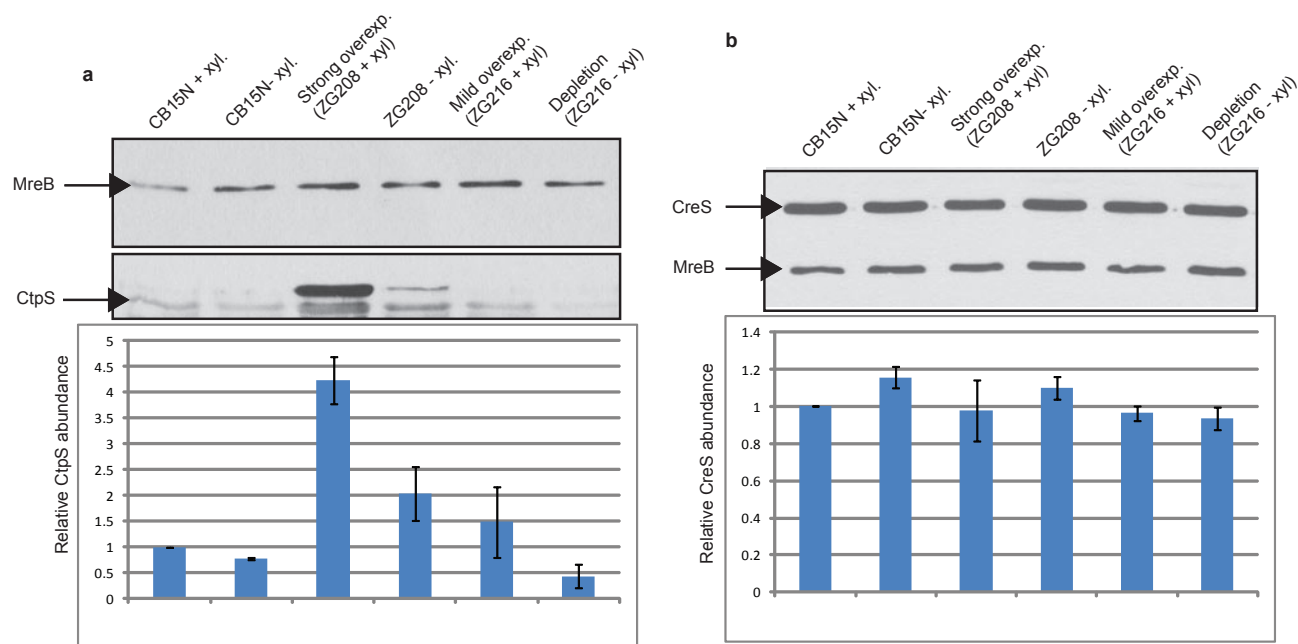


Figure S3 Abundance of CtpS and CreS in CtpS overexpression and depletion strains. **(a-b)** Abundance of CtpS **(a)** and CreS **(b)** in CtpS overexpression and depletion strains. Each panel displays a representative blot of CtpS **(a)** or CreS **(b)** abundance in strains strongly overexpressing (ZG208 grown in xylose), mildly overexpressing (ZG216 grown in xylose), and depleted (ZG216 grown without xylose) of CtpS. A strain expressing CtpS at endogenous levels (CB15N) was included as a control for xylose induction of CtpS. MreB was assayed as a loading control. α MreB

and α CreS antibodies were used at a 1:15,000 concentration, α CtpS antibodies were used at a 1:2,500 concentration. ImageJ was used to quantify CtpS and CreS abundance from multiple experiments. CtpS was detected as a doublet in all experiments; therefore CtpS levels were calculated from the intensity of both bands in the doublet. Displayed below each blot is the average of the normalized intensity of CreS and CtpS from multiple experiments. Error bars represent the standard deviation from the mean.

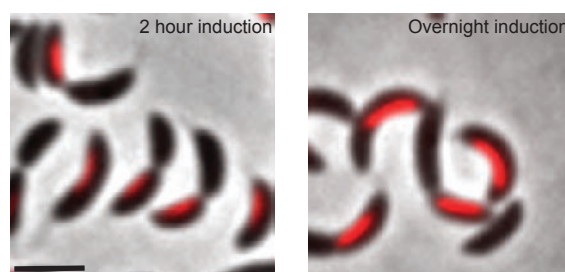


Figure S4 Overexpression of mCherry-CtpS protein. *mCherry-ctpS* expressing cells (ZG153) were grown for 2 hours in the presence of 0.03% xylose (left)

or overnight in the presence of 0.3% xylose (right) and imaged. A merged phase fluorescent image is shown. The scale bar represents 2 μ m.

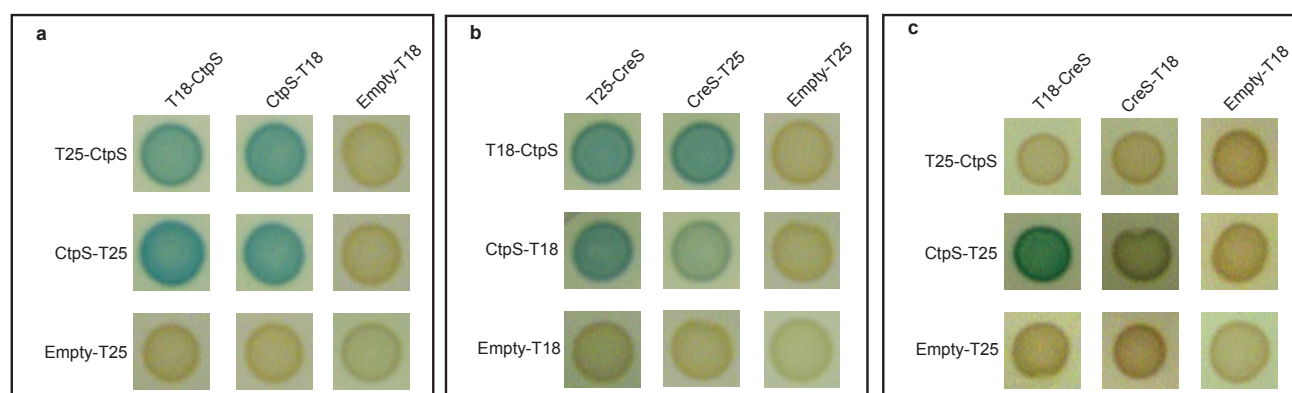


Figure S5 CtpS interacts with itself and CreS by bacterial two hybrid assay. **(a)** Bacterial two-hybrid analysis of all possible pair-wise combinations of CtpS/CtpS interactions. **(b-c)** Bacterial two-hybrid analysis of pair-wise combinations of T18 CtpS/T25 CreS **(b)** and T25

CtpS/T18 CreS **(c)** interactions. N- and C- terminal fusions of T18 and T25 to CtpS and CreS are indicated by the placement of the T18 and T25 relative to CtpS and CreS (e.g. T25-CtpS denotes an N-terminal fusion of T25 to CtpS).

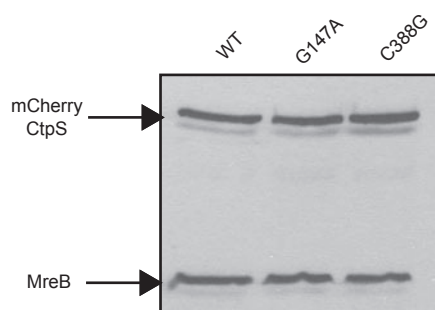


Figure S6 mCherry-CtpS protein levels in CtpS point mutation strains. Strains expressing mCherry fusions to the WT (ZG153), G147A (ZG154), and C388G (ZG155) variants of *Caulobacter* CtpS were analyzed by Western

blot using antibodies to both mCherry and MreB as a loading control. mCherry and MreB antibodies were used at 1:5,000 and 1:15,000 dilutions respectively.

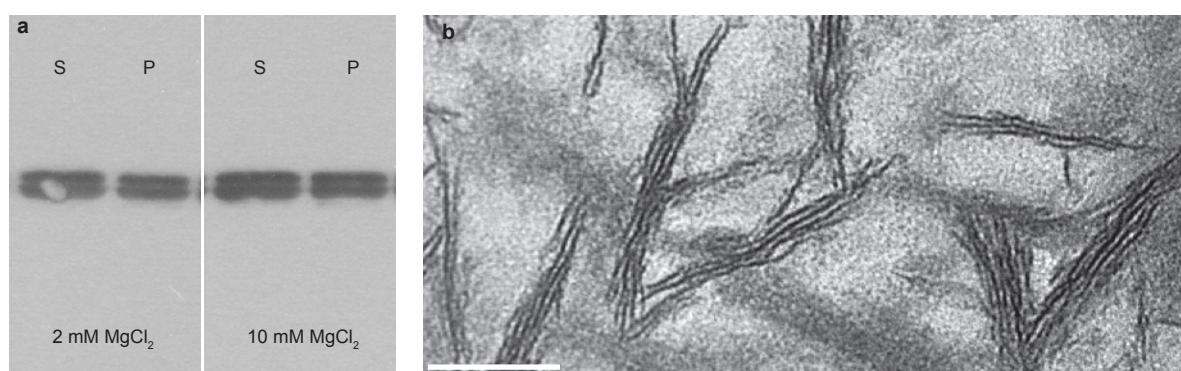


Figure S7 CtpS sedimentation and polymerization at 2 mM MgCl₂. (a) Western blot of a sedimentation assay with purified *E. coli* CtpS protein. Lanes corresponding to the supernatant and pellet fractions after ultracentrifugation at 116K x G are labeled “S” and “P” respectively. Characteristic sedimentation results for 2 mM (left) and 10 mM (right) MgCl₂

are shown. αCtpS antibodies were used at a concentration of 1:15,000 to detect CtpS protein which runs as a doublet. (b) Electron microscopy image of purified *E. coli* CtpS filaments observed *in vitro* after 10 minutes of incubation in CtpS activity buffer with 2 mM MgCl₂. The white scale bar represents 100 nm.

SUPPLEMENTARY INFORMATION

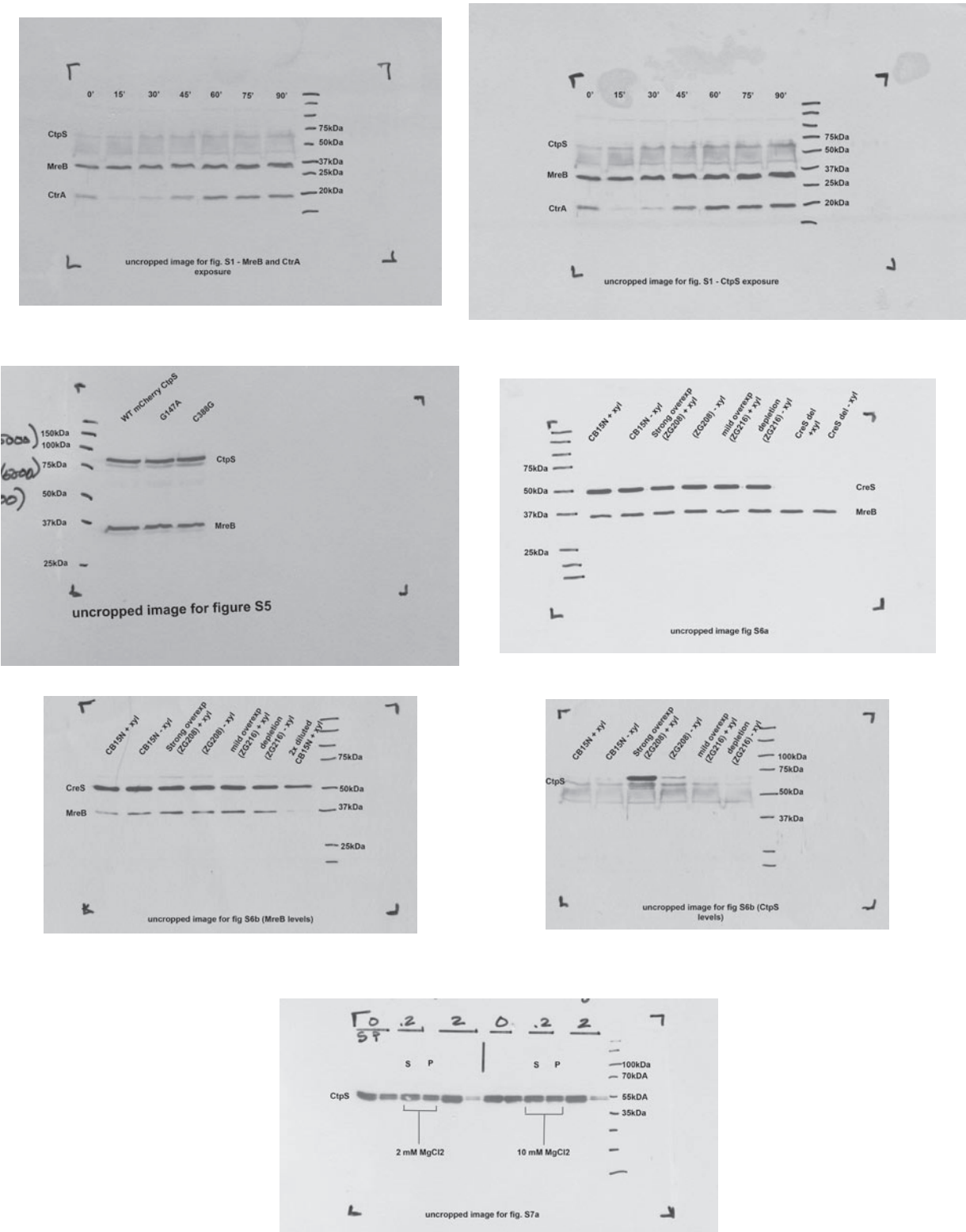


Figure S8 Full scans